REVIEW

Monitoring thiopurine metabolites in inflammatory bowel disease

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ABSTRACT

Thiopurines (azathioprine and mercaptopurine) are one of the immunosuppressive mainstays for the treatment of inflammatory bowel disease. In spite of its widespread use, thiopurine metabolism is still not fully understood, and a significant proportion of patients suffer toxicity or lack of efficacy. Different enzymatic pathways with individual variations constitute a pharmacogenetic model that seems to be suitable for monitoring and therapeutic intervention. This review is focused on current concepts and recent research that may help clinicians to rationally optimise thiopurine treatment in patients with inflammatory bowel disease.

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic and disabling inflammatory disorder that is caused by an incompletely known immunological dysregulation in a genetically predisposed patient. The main two conditions comprised in the IBD spectrum are Crohn's disease (CD) and ulcerative colitis (UC).

One of the bases of the therapeutic approach for IBD is the use of immunomodulatory drugs, in an attempt to correct the immunological disorder that causes the disease. Thiopurines, azathioprine (AZA) or mercaptopurine (MP), have been widely used as steroid-sparing agents and are indicated to maintain remission in both CD and UC, although their mechanism of action is still not fully understood. In spite of their widespread use, between 30 and 50% of the patients have to discontinue thiopurines because of adverse events or lack of efficacy.[1–8] Since efficacy and toxicity of thiopurines are thought to be related to individual variations of thiopurine metabolism, research has been focused on understanding, monitoring or even modifying the metabolic pathways of thiopurines in each particular patient.[9 10]

This article is focused on the current concepts of thiopurine metabolism and on the most recent studies that may help clinicians to optimise thiopurine treatment in patients with IBD.

THIOPURINE METABOLIC PATHWAY

The first step of AZA metabolism is its conversion into MP. This process occurs in the liver, and up to 90% of the process is non-enzymatic, although at least a small proportion of this conversion can be related to the enzyme glutathione S-transferase (GST).[10 11] As a matter of fact, deletion of GST-M1 (which determines reduced enzymatic activity) has been recently related to reduced active metabolite concentrations and reduced sensitivity to AZA in young patients.[12]

Both AZA and MP are prodrugs, which require intracellular activation by a complex multienzymatic process involving three competing pathways via three different critical enzymes (figure 1): hypoxanthine phosphoribosyl transferase (HPRT), thiopurine methyltransferase (TPMT) and xanthine oxidase (XO). HPRT converts AZA/MP into 6-thioinosine monophosphate (6TIMP), which further metabolises into the active cytotoxic metabolite 6-thioguanine nucleotide (6TGN) through a multienzymatic pathway that includes also TPMT, inosine monophosphate dehydrogenase (IMPDH) and guanosine monophosphate synthetase (GMPS). IMPDH directly converts 6TIMP to 6TGN, avoiding the intermediate 6-methylmercaptopurine ribonucleotide (6MMPR), which may have hepatotoxic effects and probably limits thiopurine phosphorylation. XO metabolises an important part of MP into the inactive agent 6-thiouric acid, and TPMT catalyses two reactions resulting in

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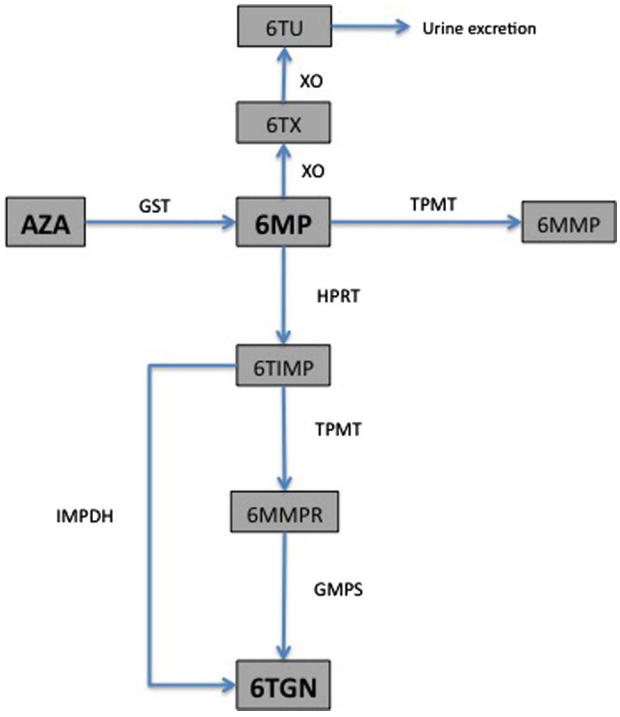

Figure 1 Thiopurine metabolic pathway.

the formation of 6-methylmercaptopurine (6MMP) and 6MMPR, which may be responsible for some toxic effects like hepatotoxicity.[9 11 13–18]

6TGN has immune modifier activity and is considered the main therapeutic metabolite of thiopurines, but may lead to myelosuppression. It is a purine antagonist that inserts within the DNA of leucocytes, and is therefore responsible for inhibiting its DNA synthesis and subsequent proliferation of T lymphocytes.[11 16 18 19] Besides that, some other immunomodulating properties that may play a role in downregulating factors involved in intestinal inflammation have been associated to 6TGN: it has been found to inhibit tumour necrosis factor related apoptosis ligand, α4-integrin in activated T lymphocytes or the expression of the Rac1 protein (implicated in T-cell proliferation), leading all of these to immunosuppression through a wide spectrum of immunosuppressive roles.[11 20 21]

TPMT is the principal enzyme in the regulation of thiopurine metabolism. The *TPMT* gene has an autosomal codominant inheritance; occasional genetic polymorphism has been described regarding the TPMT activity, which results in a trimodal distribution; those patients heterozygous or homozygous for the 'low activity' mutation gene may have a special susceptibility for myelotoxicity with thiopurine therapy. Approximately 90% of the Caucasian population is homozygous for the wild-type *TPMT* gene and have normal or high activity of this enzyme; it is a commonly held belief that this characteristic provides a safer condition regarding the intake of thiopurines. Approximately 10% and 0.5% of this population are heterozygous for intermediate enzyme activity and homozygous for the low enzyme activity, respectively. Interestingly, allelic frequency patterns also vary among ethnic groups.[22]

It is believed that low TPMT activity results in high 6TGN levels and higher associated risk of leucopenia. Therefore, patients with low TPMT activity should not receive thiopurines; if AZA or MP are considered in this kind of patients, low doses and tight hematological monitoring should be warranted.[23 24] On the other hand, high TPMT activity results in less 6TGN levels, and therefore less therapeutic efficacy, but more 6MMP, which may cause hepatotoxicity. Moreover, higher TPMT activity (>14 U/mL red blood cells (RBC)) preferentially produces 6MMP instead of 6TGN, and it could explain the lack of clinical response and increased risk of hepatotoxicity in these patients.[15 18 25–29]

MONITORING THIOPURINE METABOLITES

Traditional approach to AZA dosing is weight-based, and is usually established at approximately 2.5 mg/kg/day. Regarding MP, it is usually dosed at half of AZA, which means 1–1.5 mg/kg/day, since MP is approximately half of AZA in terms of molecular weight, and almost 90% of AZA is finally converted to MP. However, individual variations in thiopurine metabolism are the causes of interindividual differences in both therapeutic benefit and adverse events profile. These differences could be higher than 50%, in terms of therapeutic response.[4 9 15 27 29–33] Recent research in monitoring serum levels of active metabolites of thiopurines may help clinicians to optimise effectiveness and safety of thiopurine treatment.

Despite the widespread use of thiopurines and the growing knowledge about their interindividual variations in metabolism, monitoring of thiopurine metabolites is not widely used: at least two recently published surveys reveal that determination of TPMT activity (genotype or phenotype), 6TGN or 6MMP levels is used by a relatively low proportion of IBD gastroenterologist, the availability or reimbursement issues being the most relevant obstacles.[34 35]

Efficacy and safety of thiopurines

Even though 6TGN is the active metabolite of thiopurine drugs, high 6TGN levels can also be the cause of myelosuppression. Clinical benefit of thiopurine drugs has been related with serum 6TGN levels between 235 and 450 pmol/8×10^8 RBC, although the existence of a specific and truly reliable 6TGN cut-off level still remains controversial.[26 36 37] This association between 6TGN serum levels and clinical benefit has been shown with clinical response and with endoscopic improvement of CD mucosal lesions.[38]

Although some previous studies suggested that the correlation between 6TGN serum levels and weight-based thiopurine dosing might be poor, recent data have shown good positive correlation between oral

dosage of AZA and 6TGN serum levels in paediatric population.[33 39–41] Therefore, adjusting AZA dosing according to 6TGN serum levels can be a useful tool leading to improved outcomes at least in cases of inefficacy or adverse events, as it has been recently shown in a retrospectively observational study focused on the clinical usefulness of thiopurine metabolites monitoring in South Australia.[42] In spite of all of that, a recent multicentre, double-blind randomised controlled trial failed to demonstrate superiority of individualised AZA dosing based on 6TGN serum levels over classical weight-based dosage approach of AZA in patients with IBD,[43] although the size of the population studied was probably underpowered and the investigators reported inability to achieve the target 6TGN concentrations in the individualised arm.[44]

There have been many attempts to identify haematological indices as surrogate markers for therapeutic success of thiopurine, which is a useful tool to optimise thiopurines dose in clinical practice. These strategies include monitoring changes in the red cell mean corpuscular volume (MCV) or induction of leucopenia. Even though leucopenia is a well-recognised effect of thiopurines and closely related to their therapeutic mechanism, its association with therapeutic efficacy has yet to be determined. In spite of recent research, correlation between 6TGN serum levels and lymphopenia or leucopenia still remains uncertain, although a prospective observational study has recently shown that MCV>101fL reliably excluded subtherapeutic 6TGN levels.[33 41 45–47]

There are some other determinations that can be useful to understand the thiopurine metabolism of an individual patient. Genetic polymorphisms in TPMT have been related to interindividual differences in efficacy and safety. TPMT activity has been found to be inversely related to the clinical response to thiopurines, while a meta-analysis found that low TPMT activity was related to myelotoxicity but not hepatotoxicity or pancreatitis.[48] Established skewed metabolism of thiopurines that preferentially produces 6MMP rather than 6TGN is probably the major risk factor for thiopurine failure (lack of efficacy or adverse events) in patients with IBD. Identifying those patients may be a very useful prognostic tool that may help clinicians to provide adequate therapeutic approach to each individual patient.[49 50] High 6MMP levels (>5700 pmol/8×10^8 RBC) are associated with adverse events, and a retrospective study found that lack of response to AZA or MP was associated to a high 6MMP/6TGN ratio.[5 9 51]

Many dosing strategies have been proposed according to the TPMT activity, although no one is evidence-based. The classic approach stands for starting thiopurines at low doses and gradually titrating in an attempt to avoid adverse events; this strategy can be safe but long-lasting. TPMT determination may be useful to select those patients with high TPMT activity to receive weight-based full dose from the start, in a safe and also faster and cost-effective way. Appropriate dosing for patients with intermediate or low dose still remains controversial.[1 4 11 52–54]

However, the thiopurine metabolism is still not fully understood, and some recent evidence suggests that there should be alternative metabolic pathways for preferentially producing 6MMP beyond high TPMT activity.[55] Thus, some genetic variants of TPMT related to bone marrow toxicity have been recently identified; in patients carrying any of those TPMT variants, dose reduction may avoid haematological events.[56–58]

Adherence to thiopurines

Determination of 6TGN serum levels has been employed also to successfully identify non-adherent or underdosed patients. This is especially relevant in those non-responding patients: low levels of 6TGN and 6MMP may lead to strength measures to improve adherence or to increase the dose of thiopurines. Moreover, some evidence suggests that medical supervision based on the routine determination of thiopurine metabolites may improve adherence to therapy, and therefore result in better outcomes.[59 60]

Thiopurine-combined treatments

Combination of AZA or MP with aminosalicylates is very common in clinical practice.[61] Many studies have found that aminosalicylates increase 6TGN levels in a majority of the patients, although whether or not this increase has clinical significance still remains controversial. In a small prospective study carried in Asian patients, the myelotoxicity rate was 47% in the combination group, while it was only 16% in the thiopurine monotherapy group; on the other hand, a previously reported cross-sectional study including 14 545 Spanish patients showed that combination with aminosalicylates was associated with a slight increase of 6TGN levels, which was statistically significant but not clinically relevant.[25]

Therefore, if the combination of thiopurines with aminosalicylates is associated with higher probabilities of therapeutic success, higher risk of myelotoxicity or deserve a pre-emptive lower AZA dosing with a tight metabolite monitoring still remains unclear.[11 25 62 63] Probably, the answer depends on the genetic characteristic of the population considered. As a matter of fact, a recently published pilot study found that genetic polymorphisms of N-acetyl transferase could affect 6TGN levels in patients treated with thiopurines and aminosalicylates and could therefore influence the toxicity and efficacy of these drugs.[64]

Combination of thiopurines with anti-tumor necrosis factor (TNF) agents is also not uncommon. A randomised controlled trial showed that early initiation of combination therapy with AZA and infliximab was associated with better outcomes, including mucosal

healing, when compared with AZA or infliximab alone.[65] It also has been proposed that combo therapy may reduce immunogenicity to biologics and improve therapeutic outcomes. On the other hand, at least part of the added efficacy of the combination therapy is owing to increased trough levels of the biologics.[65 66]

Even though combining anti-TNF agents and thiopurines seems to be beneficial in terms of efficacy, some safety concerns about combination therapy lead clinicians to discontinue thiopurines after a period of time, but this strategy may have implications in long-term efficacy of the drug.[67 68] A recent cross-sectional study showed that 6TGN levels of only 125 pmol/8×10^8 RBC were enough to predict higher infliximab levels and the absence of antibodies to Infliximab (ATI), which is relevant since it points out the possibility of decreasing dose of AZA as an alternative to thiopurine withdrawal.[66]

In addition, combination therapy may have other benefits since some evidence suggests that direct drug interaction between AZA and infliximab may result in higher 6TGN levels.[69 70] However, a recently published small prospective study found no pharmacokinetic drug interaction between thiopurines and adalimumab.[71]

STRATEGIES TO OPTIMISE THIOPURINE TREATMENT BASED ON METABOLITES DETERMINATION

As previously stated, skewed metabolism that preferentially produces 6MMP instead of 6TGN seems to be the major cause of thiopurine failure. Dose escalation to achieve therapeutic 6TGN levels may lead to high 6MMP levels. Increased 6MMP/6TGN ratio is associated with poor therapeutic response and side effects that comprise headache, malaise, nausea, myalgia and hepatotoxicity.

A recently published retrospective study showed that dividing total daily dose of AZA or MP in thiopurine-intolerant patients with high 6MMP levels led to a significant reduction of 6MMP serum levels while maintaining 6TGN above therapeutic levels, with the resolution of the 6MMP-related adverse events.[72] These findings suggest that this dose-splitting strategy may lead to a change in the metabolism profile and can be considered as an alternative in this group of patients. Tight metabolite monitoring should be considered to avoid toxicity or underdose of thiopurines.[11]

Adding allopurinol to thiopurine therapy is another proposed alternative for those preferential 6MMP metabolisers since it has been shown to improve therapeutic 6TGN levels and decrease serum concentrations of toxic 6MMP metabolite.[73–75] The precise mechanism by which allopurinol is useful in shifting metabolites towards 6TGN production remains unclear, although some alternatives have been pointed out: first, direct inhibition of XO would result in shifting conversion to the remaining pathways, and subsequently to increased serum levels of active 6TGN metabolites; second, allopurinol supplementation would inhibit TPMT through the production of 6-thioxanthine and finally, the last proposed mechanism is an increased activity of HPRT enzyme towards the active pathway.[11 76 77]

Classic management of this combination of thiopurine plus allopurinol usually includes reducing thiopurines at least by 50% and adding 100 mg of allopurinol,

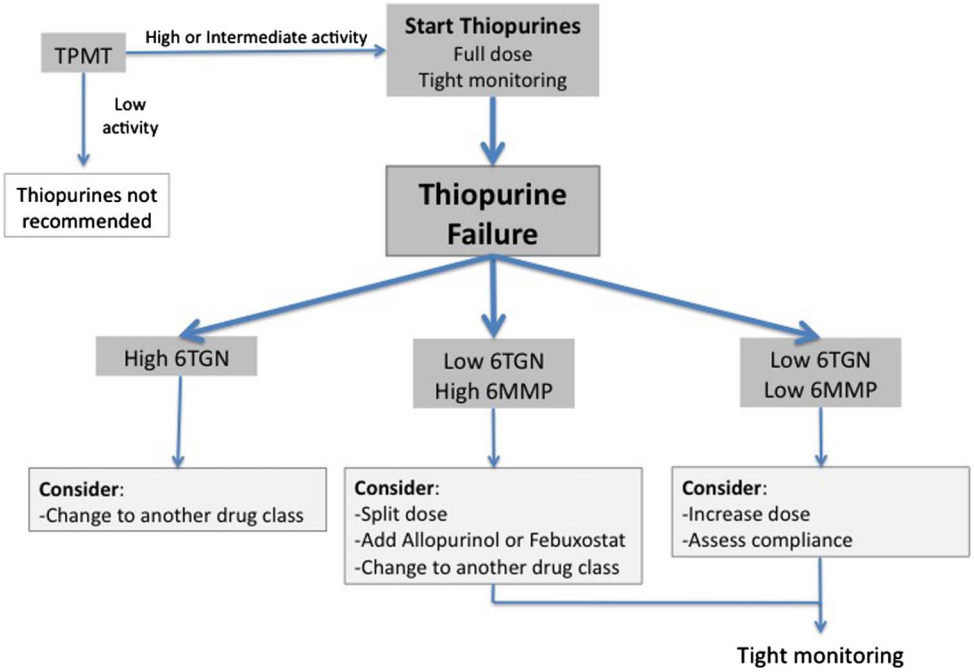

Figure 2 Proposed management of thiopurines treatment. 6MMP, 6-methylmercaptopurine; 6TGN, 6-thioguanine nucleotide; TPMT, thiopurine methyltransferase.

although recent research suggests that lower doses could also be useful and safer.[11 78] Tight metabolite and haematological monitoring is mandatory, and usually complete cell count at weekly basis during the first month and every other week during the second month is recommended.[11]

Finally, a novel XO inhibitor named febuxostat has shown, in a case study, to be useful in shifting thiopurine metabolism by preventing 6MMP production and increasing 6TGN levels.[79]

CONCLUSIONS

Thiopurine metabolism is complex and still not fully understood, but it represents a pharmacogenetic model suitable for therapeutic intervention. Current knowledge of different metabolic pathways allows clinicians to monitor and optimise the thiopurine metabolism of an individual patient. An algorithm with the proposed management of thiopurine treatment according to metabolite determination is provided (figure 2).

Determination of TPMT activity may help to identify those patients at higher risk of developing toxicity, and monitoring thiopurine metabolites may help clinicians to optimise the dose of AZA or MP at least in cases of lack of efficacy, suspect of unsatisfactory compliance or in the presence of adverse events. However, there is not enough data to support routine and systematic determination of thiopurine metabolites for every patient, which would be expensive and not always available. Combination strategies with other drugs may result in better outcomes, but require expertise and tight monitoring.

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Contributors Both authors have substantially contributed to the manuscript. YG-L: conception and design of the article, writing the manuscript, reviewed the draft and submission. JPG: conception and design of the article, reviewed the draft for important intellectual content and final approval of the version to be submitted.

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